ACS APPLIED MATERIALS & INTERFACES

Letter

www.acsami.org

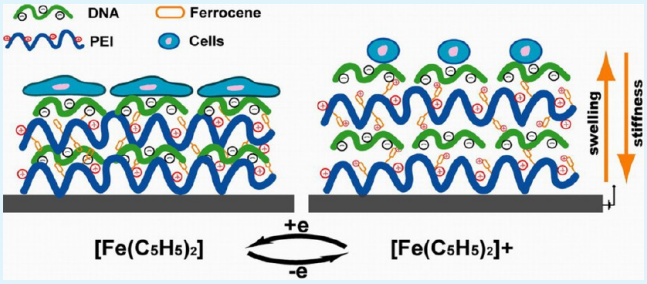
Electrochemically Controlled Stiffness of Multilayers for Manipulation of Cell Adhesion

Yi-xin Sun, Ke-feng Ren,* Jin-lei Wang, Guo-xun Chang, and Jian Ji*

MOE Key Laboratory of Macromolecule Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China

Supporting Information

ABSTRACT: Stimuli-responsive thin films attract considerable attention in different fields. Herein, an electrochemical redox multilayers with tunable stiffness is constructed through the layer-by-layer self-assembly method. The redox ferrocene modified poly(ethylenimine) play an essential role to induce multilayers' swelling/shrinking under an electrochemical stimulus, resulting reversible change of elastic modulus of the multilayers. The adhesion of fibroblast cells can be thus controlled from well spreading to round shape. Such soft multilayers with electrochemically controlled stiffness could have potentials for cell-based applications.



KEYWORDS: *cell adhesion, electrochemical redox, ferrocene, layer-by-layer self-assembly, stimuli-responsive films, stiffness*

INTRODUCTION

Stimuli-responsive thin films that are capable of physical and chemical changes on receiving an external signal have attracted considerable attention because of broad applications.[1] Responsive films that can change wettability, modulate topographical feature, and control release of drugs on stimuli, such as light, pH value, temperature, and ionic strength, have been reported.[2,3] Recently, manipulation of cellular behaviors through responsive surfaces with controlled mechanical properties is of particular interest, because it is well-proven that adhesion, migration, and differentiation of cells can be strongly affected by the stiffness of cellular microenvironment.[2,4] Herein, we report that an electrochemically responsive ultrathin film with tunable stiffness can be employed as a new strategy for manipulation of cell adhesion (see Scheme 1).

Layer-by-layer (LbL) self-assembly is a universal method for preparing functionalized polyelectrolyte multilayers (PEMs).[5,6] The procedure is simple and versatile, and it typically involves alternate deposition of oppositely charged components. Chemical and physical properties of PEMs can be tuned by external stimuli (pH, temperature, ionic strength, etc.). Recently, design of PEMs with adjustable mechanical properties has become an interesting research field for biological applications.[7] Cells generally tend to adhere and spread on a stiff surface, whereas they show a round shape on a soft surface.[4,7,8] A surface with tunable mechanical properties could be thereafter used to control the interaction between cells and substrate. In this study, the ferrocene-modified poly(ethylenimine) (PEI-Fc) is chosen as polycation to form a polyelectrolyte multilayers by LbL self-assembly. Thanks to electrochemical redox properties of ferrocene groups, the stiffness of the multilayers can be modulated when ferrocene groups undergo electrochemical redox treatment. We show that cell adhesion was thereafter controlled from well spreading to round shape on the multilayers.

EXPERIMENTAL SECTION

Materials and Reagents. Poly(ethyleneimine) (PEI, branched, M_w 25 000), ferrocene-carboxaldehyde (98%) and rhodamine-phalloidin (P1951) were purchased from Sigma-Aldrich. PEI-Fc (11 mol % grafting density of Fc) was synthesized as previously reported in our group.[9,21] Deoxyribonucleic acid (DNA, fish sperm, sodium salt) was purchased from AMRESCO. Ultrapure water was obtained from a Millipore water purification system (Milli-Q, >18 MΩ, Millipore S. A., Molsheim, France).

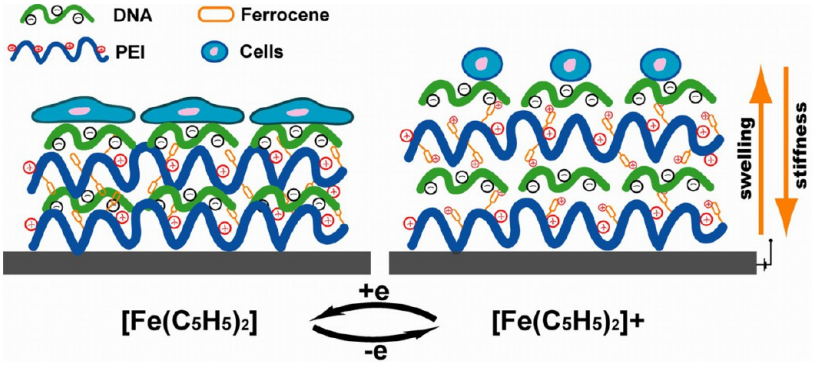
Fabrication of the (PEI-Fc/DNA) Multilayers. The $(PEI\text{-}Fc/DNA)_n$ multilayers was built up by alternating adsorptions of PEI-Fc and DNA (both at 1 mg/mL in PBS) onto substrates. Briefly, a substrate was immersed in polyelectrolyte solution for 10 min and then 2 min rinsing with PBS between steps, followed by a drying step under a stream of N_2. The process was repeated until a desired number of bilayers had been deposited.

Characterization of the (PEI-Fc/DNA) Multilayers. The growth of the (PEI-Fc/DNA) multilayers was followed by measurements of cyclic voltammetry (CV, CHI-660D electrochemistry workstation, Shanghai Chenhua, China), UV−vis spectrum (UV-vis spectrophotometer, CARY 100 BIO, USA) and quartz crystal microbalance (QCM-D, Q-Sense E4, Sweden). For CV measurement, it was carried out using a three-electrode cell. The working electrode was an Au substrates coated with the multilayers. The counter electrode was a platinum wire and the reference was a saturated calomel electrode (SCE). PBS was used as electrolyte solution. Scan rate was 50 mV/s.

Received: March 26, 2013
Accepted: May 20, 2013
Published: May 20, 2013

   dx.doi.org/10.1021/am401088w | ACS Appl. Mater. Interfaces 2013, 5, 4597−4602

Scheme 1. Schematic Representation of Manipulation of Cell Adhesion on (PEI-Fc/DNA) Multilayers; Swelling of the Multilayers Can Be Controlled by Electrochemical Treatment of Ferrocene Groups, Showing Tunable Stiffness



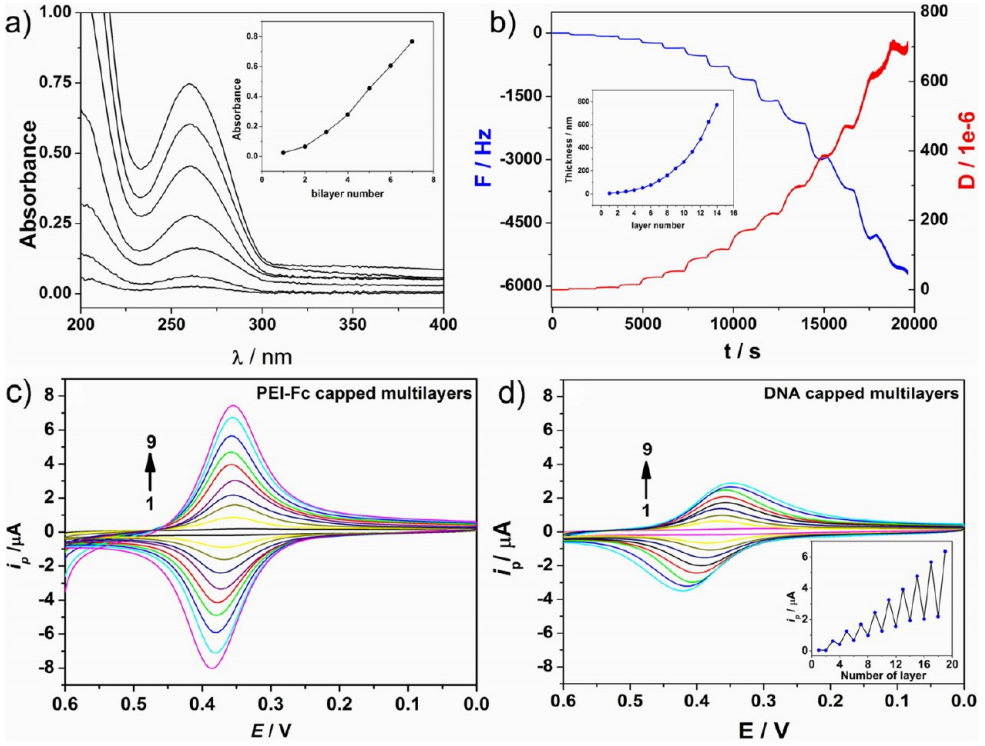


Figure 1. Characterizations of fabrication of the (PEI-Fc/DNA) multilayers. (a) UV−vis spectra of the multilayers show successive assembly of DNA (the inset shows a plot of DNA absorption at 260 nm versus bilayers number.). (b) Frequency shift and dissipation change of the multilayers as measured by using QCM-D (the inset shows a plot of multilayers' thickness versus layers number.). CV of the multilayers at different bilayers with (c) PEI-Fc and (d) DNA as the outmost layer. The inset in d shows a plot of oxidation current's peak versus layers number (odd numbers, PEI-Fc-capped multilayers; even numbers, DNA-capped multilayers). Scan rate was 50 mV/s.

Scan range was 0−600 mV versus SCE. For observation of the multilayers, the sample was rinsed with pure water carefully and dried. After that, the cross-section of the multilayers was characterized by scanning electron microscopy (SEM).

Electrochemical Quartz Crystal Microbalance (EQCM-D). Electrochemical treatments of the (PEI-Fc/DNA) multilayers were performed using an EQCM-D (Q-Sense E4 system along with the electrochemistry module (QEM 401), Sweden). The $(PEI\text{-}Fc/DNA)_4$ multilayers was assembled on a gold-coated QCM crystal (Q-Sense QSX 301). The QCM chamber worked as a three-electrode electrochemical cell with Ag/AgCl referecne electrode, Pt counter electrode, and Au-coated QCM crystal coated with the $(PEI\text{-}Fc/DNA)_4$ multilayers as the working electrode. The electrolyte was PBS buffer. A CHI-660D electrochemistry workstation (Shanghai Chenhua, China) was used for electrochemical measurements.

Electrochemical Atomic Force Microscopy (EAFM). Elastic modulus and surface morphology of the $(PEI\text{-}Fc/DNA)_{10}$ multilayers in reduction (as-prepared) and oxidation state (500 mV for different time treatments) were measured by using EAFM (Multimode IIIa AFM, Bruker, USA). The multilayers was assembled on ITO glass substrates. Electrical contact between the ITO and the electrode was made with silver paste. The measurements were carried out in the PeakForce QNM mode with a silicon nitride cantilever (spring constant 0.06 N/m, SNL, Bruker, USA). Images of Young's modulus distribution and morphological feature of the multilayers were then obtained and analyzed by using Veeco NanoScope Analysis v1.2 software.

Cell Adhesion. The $(PEI\text{-}Fc/DNA)_{10}$ multilayers were coated on ITO glass and then sterilized under UV light for 30 min prior to cells seeding. NIH/3T3 (ATCC CRL-1658) fibroblasts (1.5×10^4 cells/cm^2) were adhered on the multilayers in reduction (as-prepared) and oxidation state (electrochemical treatment for 15 min at 500 mV). Cells were adhered over night in fetal bovine serum supplemented medium (10%, DMEM, Gibco, USA). To quantify cell adhesion, we

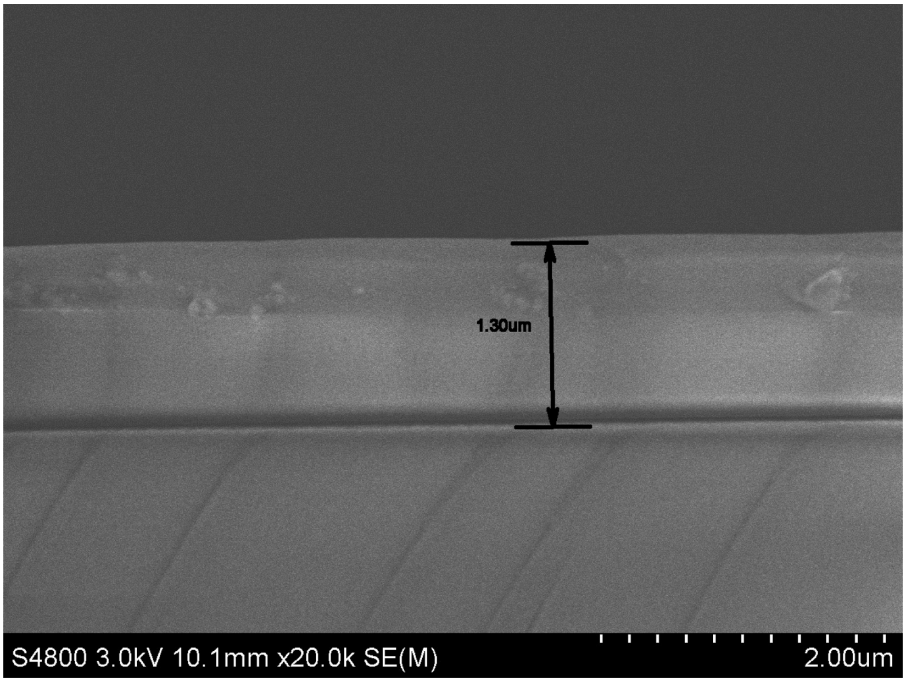


Figure 2. SEM image shows a cross-section of the $(PEI\text{-}Fc/DNA)_{10}$ multilayers.

analyzed F-actin (1:800, P1951, Sigma, USA) was stained and the images with ImageJ software v1.38.

RESULTS AND DISCUSSION

Synthesis and characterization of PEI-Fc were previously reported by our group.[9] The grafting density of ferrocene on PEI is 11 mol %. We chose DNA as anionic polyelectrolyte because of its good biocompatibility and easy commercially available. The construction of the (PEI-Fc/DNA) multilayers were firstly characterized by UV-vis spectrophotometer and QCM-D. As can been seen in Figure 1a, the plot of absorbance at 260 nm show an increase with bilayer numbers, which means that DNA has been successively incorporated into the multilayers. Figure 1b shows the changes of frequency and dissipation in the QCM-D measurement during deposition of the multilayers. Typical decrease and increase in frequency and dissipation values, respectively, were observed. These observations suggest a successful fabrication of a hydrogel like (PEI-Fc/DNA) multilayers. The multilayers exhibited exponential growth with a thickness of ~773 nm for 7 bilayers (Figure 1b, the inset). We also characterized the $(PEI\text{-}Fc/DNA)_{10}$ multilayers by SEM, showing a homogenous cross-section of the multilayers with about 1.3 μm thickness (Figure 2). Such a thick thickness could be ascribed to the "in" and "out" diffusion of polycationic molecules through the entire multilayers during each deposition (see the Supporting Information, Figure S1).[10] We further characterized electrochemical redox properties of the (PEI-Fc/DNA) multilayers by using CV. As shown in Figure 1c, during the increase in electrical potential from 0 to 600 mV, ferrocene groups $[Fe(C_5H_5)_2]$ were oxidized to $[Fe(C_5H_5)_2]^+$, resulting in oxidation peak currents from 0.05 to 6.35 and from 0.04 to 2.20 μA for PEI-Fc- and DNA-terminated multilayers with different numbers of layers, respectively. Obviously, the peak current was dependent on numbers of layers, which suggests PEI-Fc was successively deposited into the multilayers. Moreover, ferrocene groups were still electroactive both in the outer and inner layers, because both PEI-Fc and DNA terminated multilayers showed redox properties. It should be noticed that the redox peak current of DNA-terminated multilayers was much lower compared to that of PEI-Fc-terminated multilayers, because the DNA layer can inhibit electron transfer. The redox properties that are dependent on the outermost layer are often the case reported in multilayers.[11−13]

The control of PEMs' stiffness have been reported by using chemical and photo cross-linking,[14] ionic strength[15] as well as pH value.[16] In contrast to other methods, the electrical potential has advantages. It can easily be controlled with local, continuous, precise and reversible features.[17] It is known that, when applying electrochemical means, ferrocene undergoes reversible oxidation and reduction.[18] The interconversion between Fe(II) and Fe(III) states results in electrical transition of ferrocene, leading to change of microenvironments' charge density.[19] Consequently, electrochemical stimuli-responsive systems based on ferrocene have been reported.[20,21] In this study, it is reasonable to expect that the (PEI-Fc/DNA) multilayers could swell/shrink upon the electrical potential treatments. During the switch of ferrocene groups from reduction state ($[Fe(C_5H_5)_2]$) to oxidation state ($[Fe(C_5H_5)_2]^+$), the structure and ionic strength within the multilayers would be changed correspondingly. The swelling and shrinking of the $(PEI\text{-}Fc/DNA)_4$ multilayers was followed by EQCM-D. Figure 3a shows typical changes of frequency and dissipation of the multilayers that was subjected electrical potential from 0 to 600 mV. During the switch of the multilayers from reduction to oxidation state, frequency shift decreased from −680 to −830 Hz. Whereas dissipation value increased, which indicates that the multilayers became compliant during the swelling. The frequency shift subsequently increased back to −710 Hz and the dissipation value decreased during the reduction process, showing a shrinking phenomenon. This swelling/shrinking is a typical observation because of the entrance/release of counter ions for keeping the multilayers' electroneutrality and subsequent uptake/repelling

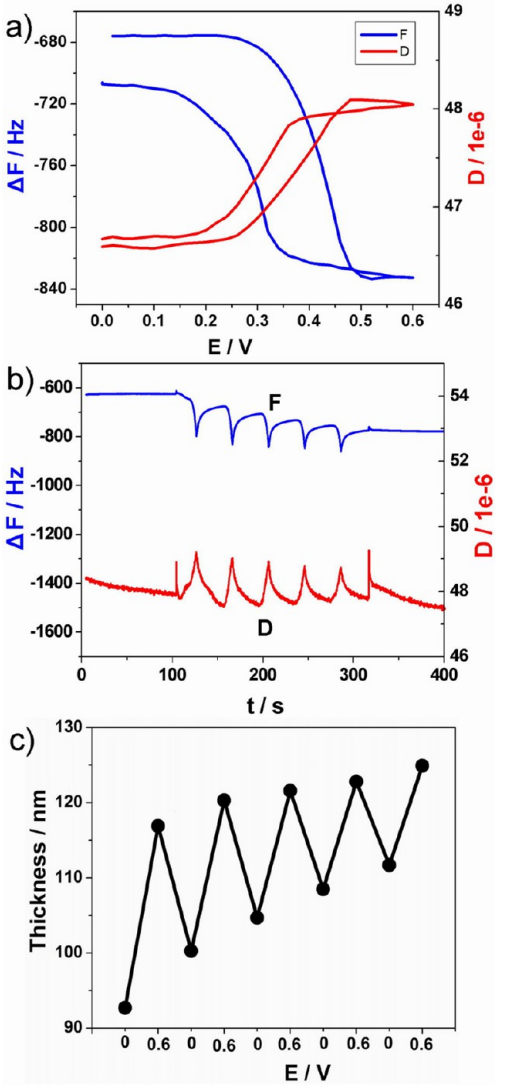


Figure 3. Electrochemical measurements of the $(PEI\text{-}Fc/DNA)_4$ multilayers. (a) Change in frequency and dissipation of the multilayers as a function of applied potential during electrochemical treatment. Scan range was 0−600 mV versus Ag/AgCl. Scan rates were 50 mV/s. (b) Frequency shift and dissipation change of the multilayers upon successive cycles of electrochemical treatments. (c) Reversible changes in thickness of the multilayers between potentials in 0 and 600 mV.

of water molecules because of osmotic pressure.[17] We then applied electrical potential on the multilayers for successive cycles (Figure 3b). One can observe the repeatable and reversible swelling/shrinking processes. Correspondingly, the thickness of the multilayers repeatedly changed on the order of 10−20% of initial thickness of multilayers (93−125 nm for the $(PEI\text{-}Fc/DNA)_4$ multilayers), as shown in Figure 3c. It is noticeable that the shrinking process didn't lead to a perfect reverse of thickness of the multilayers. Such an observation of the increase in multilayer thickness upon electrochemical redox cycles could be ascribed to the "break in" of polycations−polyanion electrostatic interactions by mobile counter ions during swelling/shrinking processes, as reported by Grumelli et al.[22] and Hammond et al.[23] In addition, the (PEI-Fc/DNA) multilayers with a relatively high thickness are like a hydrogel film. It could also increase the difficulty of shrinking process to its original thickness.

To probe the change of elastic modulus of the multilayers during the electrochemical treatment, as well as for the next cell adhesion experiment, we chose ten bilayer (PEI-Fc/DNA) multilayers. The multilayers with more than 1 μm thickness is critical in view of cell mechanosensing length scales, otherwise the substrates under multilayers could have influence on cellular behaviors.[24] The topographical feature and mechanical behavior of the $(PEI\text{-}Fc/DNA)_{10}$ multilayers, in response to electrical potential, were investigated by EAFM. The multilayers was subjected to 500 mV for different times to make the switch of multilayers from reduction to oxidation state. Figure 4A shows Young's modulus of the multilayers as a functional of

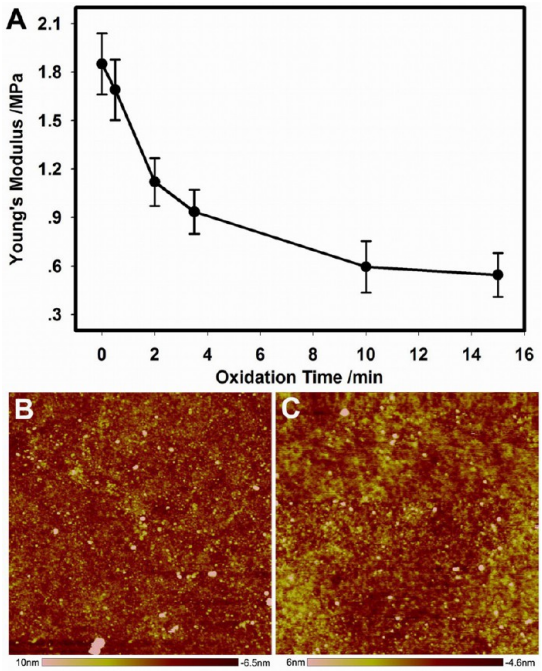


Figure 4. EAFM measurement of the $(PEI\text{-}Fc/DNA)_{10}$ multilayers. (A) Plot of change in elastic modulus of the multilayers versus oxidation time for electrochemical treatment. Applied potential was 500 mV versus Ag/AgCl. (B, C) Typical topographical images of the multilayers before and after electrochemical treatment, respectively. The size of the images is 5 μm × 5 μm.

electrochemical treatment time. After construction of the multilayers (reduction state), its elastic modulus was measured to be 1.85 ± 0.19 MPa. Upon electrochemical oxidation, stiffness of the multilayers was decreased. In 15 min treatment, its elastic modulus (oxidation state) was decreased to 0.54 ± 0.03 MPa. It is noteworthy that such the electrochemical-driven redox can be stopped at any time during the electrical potential treatment, which means that a given stiffness can be obtained. As control, such change in elastic modulus was not observed on the (PEI/DNA) multilayers (data not shown). It was previously reported that the elastic modulus values of surfaces for cell-based studies were in the range of 1 kPa to 100 MPa.[4,7,8] In particular, for the substrates based on PEMs, stiffness ranging from several hundred kPa to several MPa has been reported.[15,25−27] We thus think that the $(PEI\text{-}Fc/DNA)_{10}$ multilayers with stiffness in range of ∼2 to 0.5 MPa would be suitable for cell studies. It is worth noting that during the electrochemical treatment, there was no marked change of topographical feature of the multilayers. Very flat surfaces with the root-mean-squared (RMS) roughness of 3.39 and 5.85 nm

dx.doi.org/10.1021/am401088w | ACS Appl. Mater. Interfaces 2013, 5, 4597−4602

for reduction and oxidation state, respectively, were observed (Figure 4B, C).

Design of PEMs with adjustable mechanical properties has attracted a big interest in the field of biological applications, because PEMs is nowadays a universal method for preparing functionalized surfaces.[7,28,29] However, rarely studies have been reported on control of cells behaviors through electrochemically controlled stiffness of PEMs, as it is a key point to design and construct PEMs with good biocompatibilities and proper range of stiffness.[4,8] When cells contact with a material's surface, the first cellular event is adhesion.[30] Importantly, subsequent cellular events such as proliferation, migration, and differentiation will all be influenced by the adhesion.[31] To evaluate the influence of our multilayer system on cell adhesion, we prepared two states of the $(PEI\text{-}Fc/DNA)_{10}$ multilayers, that were in reduction state (as-prepared multilayers) and oxidation state (electrochemical oxidizing for 15 min). NIH/3T3 fibroblasts were chosen as model cells. In the case of the multilayers in oxidation state, the cell adhesion was preformed after the electrochemical treatment for avoiding the influence of electrical potential on cells. Figure 5 shows typical images of

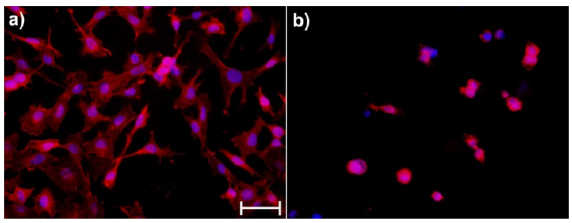


Figure 5. Cell adhesion on the $(PEI\text{-}Fc/DNA)_{10}$ multilayers in (a) reduction state and (b) oxidation state. F-actin (red) and nucleus (blue) of cells were stained. Scale bar is 50 μm.

cell adhesion on the multilayers. On the multilayers in reduction state, cells were adhered very well with higher spreading area (Figure 5a). Very long and many filopodia can be clearly observed. In contrast, cells on the oxidized multilayers appeared mostly round shape with low cells density, as shown in Figure 5b. Table 1 shows quantitative data of cell

Table 1. Quantitative Data of Cell Adhesion on the $(PEI\text{-}Fc/DNA)_{10}$ Multilayers in the Reduction State and Oxidation States (the multilayers was applied potential of 500 mV for 15 min)

multilayer type	cell density (cells/mm^2)	spreading area (μm^2/cell)	circularity
reduction state	549.8 ± 49.3	912.1 ± 88.9	0.33 ± 0.03
oxidation state	156.5 ± 18.6	233.2 ± 17.6	0.75 ± 0.06

adhesion. These data suggest that cell adhesion can be easily controlled by using the $(PEI\text{-}Fc/DNA)_{10}$ multilayers. Generally speaking, cell adhesion could be modulated by chemistry, topographical feature, and mechanical properties of a surface.[7,32] Whereas in this (PEI-Fc/DNA) multilayers, we already showed that the multilayer surface is very flat and the electrochemical treatment did not change the topographical feature (Figure 4b, c). With regard to the chemistry properties, we have prepared the multilayers with DNA as the outmost layer. It could avoid the influence of charge on cell adhesion and the electrochemical treatment should have minimally affected on the surface chemistry. We carried out X-ray photoelectron spectroscopy (XPS) measurement and confirmed that the multilayers' surface have kept the same chemistry properties during the electrochemical treatment (see the Supporting Information, Figure S2). Therefore, we have identified that difference in stiffness was the main reasonable factor that influenced cell adhesion.

CONCLUSIONS

In summary, we report the (PEI-Fc/DNA) multilayers with controlled stiffness by using electrochemical mean. Upon the electrical potential, the PEI-Fc was switched between reduction and oxidation states, leading to swelling/shrinking of the multilayers. The Young's modulus of the multilayers can be thereafter controlled. The multilayers was thus used to modulate cell adhesion. The (PEI-Fc/DNA) multilayers with appropriate range of mechanical changes upon electrochemical-driven redox could be applied to the active surfaces for tissue engineering, regenerative medicine, as well as biomedical devices, thereby enhancing their cell-based applications.

ASSOCIATED CONTENT

Supporting Information

Confocal microscopy measurement of PEI-Fc diffusion within the multilayers and XPS measurement of the $(PEI\text{-}Fc/DNA)_{10}$ multilayers with and without electrochemical treatment. This material is available free of charge via the Internet at http://pubs.acs.org/.

AUTHOR INFORMATION

Corresponding Author

*E-mail: renkf@zju.edu.cn (K.-f.R.); jijian@zju.edu.cn (J.J.). Tel./Fax.: +86 571 87953729.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Financial support from the National Natural Science Foundation of China (50830106, 21174126, and 51103126), China National Funds for Distinguished Young Scientists (51025312), the National Basic Research Program of China (2011CB606203), Open Project of State Key Laboratory of Supramolecular Structure and Materials (SKLSSM201316), and Research Fund for the Doctoral Program of Higher Education of China (20110101110037, 20110101120049, 20120101130013) is gratefully acknowledged.

REFERENCES

(1) Stuart, M. A. C.; Huck, W. T. S.; Genzer, J.; Muller, M.; Ober, C.; Stamm, M.; Sukhorukov, G. B.; Szleifer, I.; Tsukruk, V. V.; Urban, M.; Winnik, F.; Zauscher, S.; Luzinov, I.; Minko, S. *Nat. Mater.* **2010**, *9*, 101−113.
(2) Mitragotri, S.; Lahann, J. *Nat. Mater.* **2009**, *8*, 15−23.
(3) Mendes, P. M. *Chem. Soc. Rev.* **2008**, *37*, 2512−2529.
(4) Discher, D. E.; Janmey, P.; Wang, Y. L. *Science* **2005**, *310*, 1139−1143.
(5) Decher, G. *Science* **1997**, *277*, 1232−1237.
(6) Lavalle, P.; Voegel, J. C.; Vautier, D.; Senger, B.; Schaaf, P.; Ball, V. *Adv. Mater.* **2011**, *23*, 1191−1221.
(7) Boudou, T.; Crouzier, T.; Ren, K.-F.; Blin, G.; Picart, C. *Adv. Mater.* **2010**, *22*, 441−467.
(8) Levental, I.; Georges, P. C.; Janmey, P. A. *Soft Matter* **2007**, *3*, 299−306.

(9) Zhu, L.-Z.; Shangguan, Y. G.; Sun, Y. X.; Ji, J. A.; Zheng, Q. A. *Soft Matter* **2010**, *6*, 5541−5546.
(10) Picart, C.; Mutterer, J.; Richert, L.; Luo, Y.; Prestwich, G. D.; Schaaf, P.; Voegel, J. C.; Lavalle, P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 12531−12535.
(11) Calvo, E. J.; Forzani, E.; Otero, M. *J. Electroanal. Chem.* **2002**, *538*−*539*, 231−241.
(12) Calvo, E. J.; Wolosiuk, A. *J. Am. Chem. Soc.* **2002**, *124*, 8490−8497.
(13) Hodak, J.; Etchenique, R.; Calvo, E. J.; Singhal, K.; Bartlett, P. N. *Langmuir* **1997**, *13*, 2708−2716.
(14) Vazquez, C. P.; Boudou, T.; Dulong, V.; Nicolas, C.; Picart, C.; Glinel, K. *Langmuir* **2009**, *25*, 3556−3563.
(15) Blacklock, J.; Vetter, A.; Lankenau, A.; Oupicky, D.; Mohwald, H. *Biomaterials* **2010**, *31*, 7167−7174.
(16) Mermut, O.; Lefebvre, J.; Gray, D. G.; Barrett, C. J. *Macromolecules* **2003**, *36*, 8819−8824.
(17) Zahn, R.; Voros, J.; Zambelli, T. *Curr. Opin. Colloid & Interf. Sci.* **2010**, *15*, 427−434.
(18) Merchant, S. A.; Glatzhofer, D. T.; Schmidtke, D. W. *Langmuir* **2007**, *23*, 11295−11302.
(19) Nishihara, H. *Adv. Inorg. Chem.* **2002**, *53*, 41−86.
(20) Casas-Solvas, J. M.; Ortiz-Salmeron, E.; Fernandez, I.; Garcia-Fuentes, L.; Santoyo-Gonzalez, F.; Vargas-Berenguel, A. *Chem.—Eur. J.* **2009**, *15*, 8146−8162.
(21) Zhu, L.-Z.; Zhou, W.-B.; Ji, J. *J. Nanopart. Res.* **2010**, *12*, 2179−2187.
(22) Grumelli, D.; Bonazzola, C.; Calvo, E. J. *Electrochem. Commun.* **2006**, *8*, 1353−1357.
(23) Schmidt, D. J.; Cebeci, F. C.; Kalcioglu, Z. I.; Wyman, S. G.; Ortiz, C.; Van Vliet, K. J.; Hammond, P. T. *ACS Nano* **2009**, *3*, 2207−2216.
(24) Sen, S.; Engler, A. J.; Discher, D. E. *Cell. Mol. Bioeng.* **2009**, *2*, 39−48.
(25) Ren, K.-F.; Fourel, L.; Rouviere, C. G.; Albiges-Rizo, C.; Picart, C. *Acta Biomater.* **2010**, *6*, 4238−4248.
(26) Ren, K.-F.; Crouzier, T.; Roy, C.; Picart, C. *Adv. Func. Mater.* **2008**, *18*, 1378−1389.
(27) Grohmann, S.; Rothe, H.; Frant, M.; Liefeith, K. *Biomacromolecules* **2011**, *12*, 1987−1997.
(28) Lin, Q. K.; Ren, K.-F.; Ji, J. *Colloids Surf., B* **2009**, *74*, 298−303.
(29) Ma, Y.; Zhang, Y.; Wu, B.; Sun, W.; Li, Z.; Sun, J. *Angew. Chem., Int. Ed.* **2011**, *50*, 6254−6257.
(30) Ratner, B. D.; Hoffman, A. S.; Schoen, F. J.; Lemons, J. E. *Biomaterials Science: An Introduction to Materials in Medicine*; Academic Press: New York, 2004.
(31) Rehfeldt, F.; Engler, A. J.; Eckhardt, A.; Ahmed, F.; Diseher, D. E. *Adv. Drug Delivery Rev.* **2007**, *59*, 1329−1339.
(32) Stevens, M. M.; George, J. H. *Science* **2005**, *310*, 1135−1138.